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NOTES

Yellow Blotch of *Pleurotus ostreatus*

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Yellow blotch disease of the oyster mushroom (*Pleurotus ostreatus*) was first observed in a commercial mushroom farm in California in 1983. The disease, caused by *Pseudomonas agarici*, is characterized by primordia, with yellow droplets on their surface, which become stunted, yellow to orange, and deformed as they mature.

The oyster mushroom (*Pleurotus ostreatus*) Jacq. ex Fr.) has been cultivated in large quantities in Japan for several years. Commercial production has increased dramatically during the past few years in Europe, Asia, and the United States. Today, *P. ostreatus* is the second most important commercially grown mushroom in Europe, exceeded only by *Agaricus bisporus* (Lange) Imbach (9). The demand for increased cultivation of the oyster mushroom has stimulated research efforts concerning its biology, genetics, and cultivation (1–5, 8). Despite such efforts, very little information about diseases of the oyster mushroom has been reported. Recently, a fungal disease of the oyster mushroom, called dry bubble, has been reported (10). This paper describes a disease of the oyster mushroom known as yellow blotch, a bacterial disease caused by *Pseudomonas agarici*.

Isolation of causal organism. Depending upon their size, a single basidiocarp or a piece approximately 2.5 cm^2 was placed in a 2.5% solution of sodium hypochlorite for 1 min and was thoroughly rinsed in sterile distilled water. Intact basidiocarps or pieces were placed into sterile plastic petri dishes containing 1 ml of sterile distilled water and cut into small pieces by using a sterile scalpel blade. A sterile Pasteur pipette was used to transfer 1 drop of the suspension to a plate of nutrient agar (Difco Laboratories, Detroit, Mich.). The inoculum was allowed to dry for approximately 30 min at room temperature, was streaked for isolation, and was incubated at 26°C for 48 h.

Methods for characterization of causal organism. Yeast mannitol agar and yeast mannitol agar with $CaCO_3$ were prepared by the method of Vincent (17); 0.05% yeast extract (Difco) and 0.3% (wt/vol) $CaCO_3$ were used to neutralize any excess acid. Potato glucose (Difco) agar plates were prepared according to the directions of the manufacturer. The biochemical reactions were interpreted from API 20E test strips (Analytab Products, Plainview, N.Y.) for differentiation of gram-negative bacteria. Supplemental tests including benzoate, gluconate, lecithinase, fructose, maltose, and lactose reactions were carried out by the methods described previously (7). The carbohydrate media were examined for production of acid, gas, or both over a 7-day period at 28°C. Fluorescence was checked at 2,537 Å (253.7 nm) on *Pseudomonas* agar F (Difco). Colony morphology was observed after 5 to 6 days of incubation at 28°C on nutrient agar.

Pathogenicity studies. The inoculum was prepared by adding a portion of a colony from a nutrient agar plate to a tube containing 4 ml of sterile distilled water. Next it was adjusted to yield an optical density of 0.1. Using a sterile Pasteur pipette, we placed 1 drop of the adjusted inoculum onto each of several primordia of *P. ostreatus*. In another set of experiments, the adjusted inoculum was applied as an atomized spray. The oyster mushrooms were incubated at 60 to 65°F (15 to 18°C) at a relative humidity of 85 to 95% and were continuously illuminated by fluorescent light. Developing sporocarps were monitored daily for up to 10 days for the appearance of symptoms. Negative controls consisting of sterile distilled water were used whenever isolates were tested. Those isolates which caused formation of symptoms were selected for further characterization.

The primary strain of *P. ostreatus* on on which these investigations were performed has a characteristic macromorphology. When primordia develop on a vertical or nearly vertical surface, the resultant sporocarps grow semilaterally, forming tight clusters. The pileus is convex in profile and suborbicular to flabelliform when viewed from above (Fig. 1).

The first indication of infection was the production of droplets of clear yellow fluid on the cluster surface. Primordia infected early in their development gave rise to sporocarps which deviated from unaffected mushrooms in the following ways. The stipes tended to recurve near the base rather than near the apex; and the resultant sporocarps had an upright rather than a lateral habit. Fewer primordia developed, while the stipes of those that did were of such uneven length that the resultant sporocarp clusters were loose and open. The stipes sometimes felt fibrous or gritty when compressed between the fingers. Stipe diameter was sometimes reduced, producing a spindly appearance. In severe cases the mushrooms were deformed, disoriented, bright yellow to orange, more brittle than usual, and somewhat stunted (Fig. 2). Flushes of mushrooms developing after symptomatic ones may be asymptomatic or almost so, or may be as or more severely symptomatic than their predecessors.

Characterization of causal organism. Colonies of the six pathogenic strains isolated were buff colored, semiopaque, 2

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FIG. 1. Uninfected sporocarps of *P. ostreatus*. Magnification, ×1.

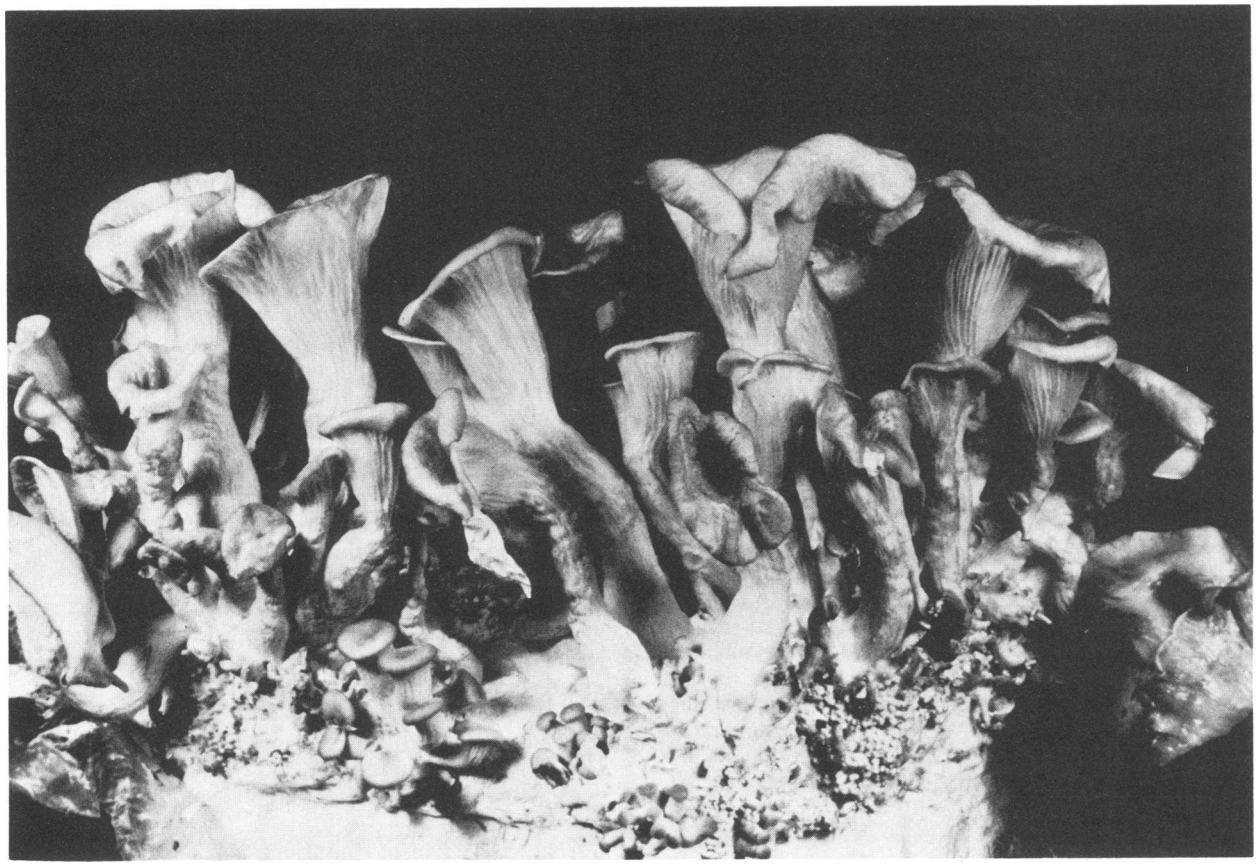

FIG. 2. Sporocarps of *P. ostreatus* showing symptoms of yellow blotch disease. Magnification, ×1.

to 5 mm in diameter, circular, pulvionate, and entire. In nutrient broth cultures, the organism forms a thin membranous pellicle at the surface. The isolates were gram-negative, rod-shaped, motile bacteria.

Oxidase, catalase, and Voges-Proskauer reactions were positive, and nitrate reduction, starch hydrolysis, and esculin hydrolysis were negative. Gluconate, benzoate, and citrate were all utilized.

Acid was produced from glucose, arabinose, fructose, and maltose. No acid was produced from rhamnose, sucrose, lactose, maltose, inositol, sorbitol, mannose, or melibiose.

The results of these tests were consistent with those obtained by Young (18), and therefore the isolates were identified as *Pseudomonas agarici*.

This is the first report of *Pseudomonas agarici* as a pathogen for *P. ostreatus*. This organism has been reported to cause drippy gill of *A. bisporus* (12, 18). Similar diseases of the cultivated agaric, including mummy disease (14, 16) and brown blotch (13, 15), have been reported, but the organisms responsible for these diseases are poorly understood.

A definite relationship between relative humidity, formation rate and severity of symptoms was observed. As the humidity increased to a level above 95%, the rate of symptom formation and severity of symptoms increased significantly. Other investigators have noted a similar relationship from brown blotch disease (6, 11). Cultivation of substrates including sawdust, wood chips, and shredded newspaper were negative for *P. agarici*. A water sample from the evaporative cooling system was positive for *P. agarici*.

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